Cell, Vol. 90, 951–957, September 5, 1997, Copyright ©1997 by Cell Press

Subcellular Distribution of Actively Partitioning F Plasmid during the Cell Division Cycle in E. coli

Hironori Niki and Sota Hiraga
Department of Molecular Cell Biology
Institute of Medical Embryology and Genetics
Kumamoto University School of Medicine
4-24-1 Kuhonji
Kumamoto, Kumamoto 862
Japan

Summary

F plasmid is partitioned with fidelity to daughter cells during cell division cycle owing to two *trans*-acting genes, *sopA* and *sopB*, and a *cis*-acting site, *sopC*. We visualized the subcellular distribution of mini-F-plasmid molecules by fluorescence in situ hybridization. Mini-F-plasmid molecules having the *sopABC* segment were localized at midcell in newborn cells. Replicated plasmid molecules migrated to cell positions 1/4 and 3/4 without coupling with cell elongation and were tethered to these positions until completion of cell division. In contrast, molecules of a mini F plasmid lacking the *sopABC* segment were distributed randomly in spaces not occupied by nucleoids. The *sopABC* system caused replicated plasmid molecules to be positioned and tethered at the cell quarter sites.

Introduction

Low copy number plasmids, such as the F plasmid (the fertility factor of E. coli) and the P1 prophage, which is replicated as an extrachromosomal element in lysogenic cells, are stably maintained in host cells during cell proliferation through a mechanism for active partitioning (Nordström and Austin, 1989; Hiraga, 1992). The copy number of the F and P1 plasmids is stringently controlled, giving only one or two copies per cell (Scott, 1984). These plasmids have their own partitioning system controlled by the plasmid-encoded gene products, F SopA/P1 ParA and F SopB/P1 ParB proteins and a plasmid-specific DNA sequence that acts as a centromere-like site on each plasmid. The structure and function of these partition proteins are similar in F and P1 plasmids (Motallebi et al., 1990; Hiraga, 1992). Recently, bacterial chromosomal genes homologous to these plasmid genes have been found in Bacillus subtilis, Pseudomonas putida (Ogasawara and Yoshikawa, 1992), and Caulobacter crescentus (Mohl and Gober, 1997). These chromosomal genes form an operon and might play roles for partitioning of the bacterial chromosome into both daughter cells (Lin et al., 1997; Mohl and Gober, 1997; Webb et al., 1997). It is therefore important to analyze precisely the molecular mechanism of plasmid partitioning as a model system to elucidate the chromosome partitioning system.

In the F plasmid, the *sopA* and *sopB* gene products and the *cis*-acting *sopC* site that contains twelve 43-bp direct repeats are essential for the partitioning system (Ogura and Hiraga, 1983b; Mori et al., 1986). The *sopA* and *sopB* genes form an operon, expression of which is transcriptionally autoregulated by the cooperative function of SopA and SopB proteins (Mori et al., 1989). Moreover, the binding of SopA to the promoter region of the *sopAB* operon is stimulated by SopB in vitro. The harmonious expression of these proteins is essential for accurate partitioning of the F plasmid (Kusukawa et al., 1987). The purified SopB protein binds to the *sopC* DNA region (Mori et al., 1989; Watanabe et al., 1989; Hanai et al., 1996). The purified SopA protein has an activity of Mg^{2+}-dependent ATPase (Watanabe et al., 1992) that is stimulated by the addition of double-stranded DNA and SopB protein. Similar results were described for the ParA and ParB proteins of P1 plasmid (Davis and Austin, 1988; Funnell, 1988; Davis et al., 1992, 1996). Recently, it was reported that the ATPase activity of P1 ParA is not only essential for repression of the *parAB* operon but also for some steps in the partitioning process itself (Davis et al., 1996). Members of the ATPase family, including F SopA and P1 ParA, have been found in other plasmids (Motallebi et al., 1990).

The *sopB* homolog, *spo0J*, in B. subtilis was originally identified as a gene regulating sporulation (Mysliwiec et al., 1991). A *spo0J* mutant causes production of a significant proportion of anucleate cells and is blocked at the onset of sporulation (Ireton et al., 1994). The *soj* gene, homologous to the F *sopA* gene and in an operon with *spo0J*, suppresses the sporulation block of the *spo0J* mutation (Ireton et al., 1994). The replication origin regions are localized preferentially near each cell pole in vegetatively growing cells (Webb et al., 1997). The bipolar localization of the Spo0J protein was observed by immunofluorescence microscopy, and Spo0J binds to the DNA site in the origin-proximal third of the chromosome (Lin et al., 1997). The ParA and ParB proteins of C. crescentus are localized at poles of the cells in a stage of the cell cycle (Mohl and Gober, 1997). The ParB protein of C. crescentus binds in vitro specifically to a sequence downstream of the *parAB* operon, near the replication origin of the C. crescentus chromosome. Overproduction of ParA and ParB inhibits both cell division and chromosome partitioning (Mohl and Gober, 1997). Thus, the members of the ParA and ParB families play roles in chromosome partitioning in C. crescentus and B. subtilis.

F and P1 plasmids are stably maintained in a *mukB* null mutant (Ezaki et al., 1991; Funnell and Gagnier, 1995) that is defective in chromosome partitioning and consequently produces anucleate cells (nucleoidless cells) upon cell division (Niki et al., 1991). Moreover, the F plasmid is stably maintained in *mukF* and *mukE* null mutants, which are also defective in chromosome partitioning (Yamanaka et al., 1996). F and P1 plasmids are partitioned to both nucleate and anucleate cells in the *mukB* mutant (Ezaki et al., 1991; Funnell and Gagnier, 1995). Therefore, plasmid partitioning is not mediated by direct attachment between the plasmid and the chromosome, and the *mukF*, *mukE*, and *mukB* gene products are not essential for active partitioning of these plasmids.

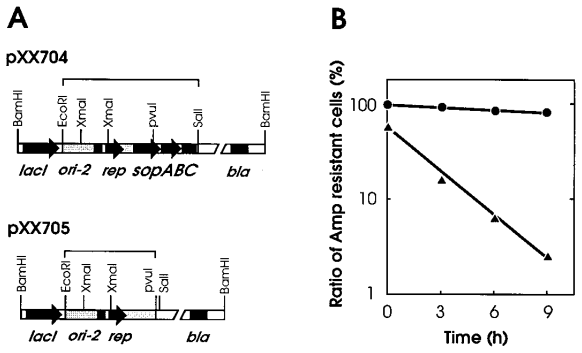

Figure 1. Structure and Stability of Mini F Plasmids pXX704 and pXX705

(A) Diagrams of structures of the mini F plasmids. Shaded region indicates the DNA segment derived from the F factor (Kline, 1985). (B) Cells harboring pXX704 and pXX705 were grown in L medium with 20 μg/ml ampicillin at 37°C and then transferred to nonselective L medium without ampicillin at zero time. Doubling time was 26 min. Closed circle, pXX704. Closed triangle, pXX705.

In a hypothetical model for the partitioning of the P1 plasmid (Austin and Abeles, 1983; Austin and Nordström, 1990), plasmid-encoded partition proteins bind specifically to a *cis*-acting site and promote pairing of plasmid DNA molecules via dimerization of the proteins. The paired protein–plasmid DNA complexes associate with a specific cellular site(s) on the future septation plane. The septum is formed between the paired complexes, so the complexes are separated from each other by septation and partitioned into the daughter cells. Observation of the subcellular localization of plasmid DNA molecules in host cells is essential to verify this model of plasmid partitioning.

We report here the subcellular distribution of the mini F plasmid during the cell division cycle, using fluorescence in situ hybridization (FISH) to visualize specifically plasmid molecules in the fixed cells. We have found that the mini-F-plasmid molecule is localized at midcell in newborn cells and that replicated plasmid molecules are positioned at the 1/4 and 3/4 sites in the cell length and tethered at these positions until completion of cell division. The positioning and tethering of the plasmid molecules at these specific cellular positions depends on the *sopABC* system.

Results

Direct Visualization of Mini-F-Plasmid DNA Molecules by FISH

Two types of derivative of the mini F plasmid, pXX704 and pXX705, were used in this study (Figure 1A). Both plasmids have the replication origin, *ori-2*, and the *repE* gene essential for replication initiation (Scott, 1984). Plasmid pXX704 carries the *sopA* and *sopB* genes and the *sopC* DNA region, whereas plasmid pXX705 lacks the *sopABC* segment. When E. coli cells harboring pXX704 or pXX705 were incubated in nonselective medium without ampicillin, plasmid pXX704 was stably maintained in progeny cells and 80% of the cells retained the plasmid after 20 generations (9 hr). In contrast, pXX705 was unstably maintained in progeny cells, and

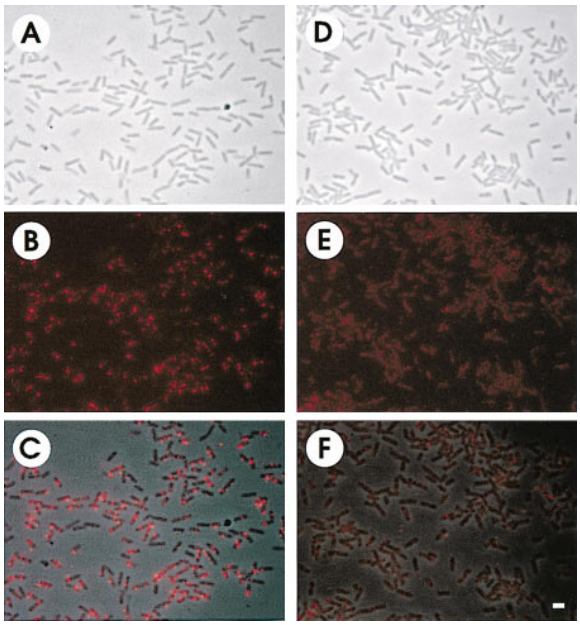

Figure 2. Observation of pXX704 Plasmid DNA Molecules by Fluorescence Microscopy

Cells were fixed and treated with the Cy3-labeled DNA probe as described in Experimental Procedures. (A–C) Cells harboring pXX704. (D–F) Plasmid-free cells. (A and D) Phase contrast micrographs. (B and E) Fluorescence micrographs for Cy3. The gain of the micrograph of plasmid-free cells (E) was 10-fold higher than that of cells harboring pXX704 (B). (C and F) Combined images of the phase contrast micrograph and fluorescence micrograph. Scale bar indicates 1 μm.

97% of cells lost the plasmid in 20 generations (Figure 1B). These results confirmed that the *sopABC* segment causes stable maintenance of the mini F plasmid in progeny cells (Ogura and Hiraga, 1983b).

To observe the subcellular localization of plasmid molecules, we used a modified method of FISH using a pXX704 DNA probe labeled with the fluorescence compound Cy3-dCTP. We detected an apparent red fluorescence focus or foci in the cells harboring pXX704 (Figure 2B), but no significant fluorescence focus in plasmid-free cells (Figure 2E). One or two fluorescence foci were observed in cells harboring pXX704 (Figure 2C), consistent with the copy number of the mini F plasmid, which is one or two copies per cell. Similar results were obtained using the pXX704 DNA probe labeled with fluorescein-11-dUTP (data not shown).

Subcellular Distribution of the pXX704 Plasmid

We observed pXX704-harboring cells by FISH. Some short cells in cell length (1.2–1.7 μm, $\mu = 1.49$ μm) had a single fluorescence focus in the center area of the cell (Figure 3Aa). Other short cells (1.4–1.7 μm, $\mu = 1.52$ μm) had two foci that were closely localized to each other (Figure 3Ab) or separated (Figures 3Ac and 3Ad). Long cells (1.8–2.6 μm, $\mu = 2.17$ μm) had two fluorescence foci localized nearly at the cellular positions 1/4 and 3/4 (Figures 3Ae and 3Af). Dividing cells with a constriction at midcell also had two fluorescence foci at positions 1/4 and 3/4 (Figure 3Ag). Figure 4A shows

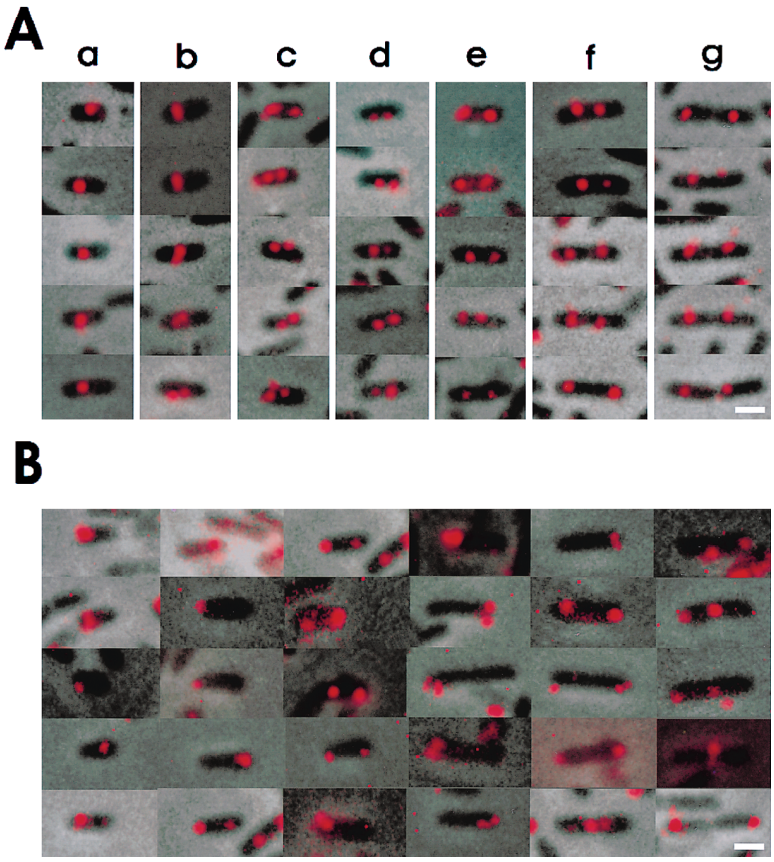

Figure 3. Subcellular Localization of Mini-F-Plasmid DNA Molecules by Fluorescence Microscopy

(A) Combined images of the phase contrast micrograph and fluorescence micrograph of the stable plasmid pXX704.

(B) Combined images of the phase contrast micrograph and fluorescence micrograph of the unstable plasmid pXX705.

Scale bar indicates 1 μm.

the subcellular localization of pXX704 DNA molecules hybridized with the DNA probe labeled with Cy3 and localization of nucleoids stained by DAPI. Red fluorescent foci of pXX704 were localized within blue fluorescent nucleoids in all cells in various stages of the cell division cycle. There were red foci that did not touch any cells at low frequency. They are probably free probe molecules that remained on a poly-L-lysine–coated glass slide. This type of foci was omitted in the statistical experiments we discuss below. We counted long cells with a deep septum constriction as one cell in the statistical experiments.

To determine precisely the subcellular distribution of pXX704 molecules, we measured the distance from one cell pole to the center of each red fluorescence focus in a number of cells (Figure 5). In cells having a single focus, the focus was localized at the central region of the cell (i.e., within the nucleoid space), but not in the cytosol spaces of the cell poles (Figures 5A and 5B). The average cell length of cells with a single focus was 1.66 ± 0.10 μm, suggesting that these cells were newborn or in early stages of the cell division cycle. On the other hand, in cells with two fluorescence foci, one focus was localized at the 1/4 position and the other at the 3/4 position (Figures 5C and 5D). It should be noticed that two foci were already separated from each other and localized at the 1/4 and 3/4 positions even in the short cells of 1.0–1.5 μm (Figure 5C). Moreover, only a few cells with two closely located foci, as shown in Figure 3Ab, were observed (Figure 5C).

Random Distribution in Cytosol Spaces of Plasmid pXX705 Lacking the *sopABC* Segment

We observed the subcellular localization of the unstable plasmid pXX705, lacking the *sopABC* segment, by the same method of FISH. Fluorescence foci of the pXX705 DNA molecules were localized at one or both cell poles (Figure 3B). As shown in Figures 4B, 6A, and 6B, the fluorescence focus was mainly localized in a cytosol area of a cell pole, that was not occupied by the chromosomal DNA, in cells having a single focus. In cells having two fluorescence foci, one focus was localized in a cytosol area of a cell pole and the other focus was localized at the same pole or the opposite pole (Figures 4B, 6C, and 6D). In long cells, 2–3 μm in length, two separate nucleoids were present and two fluorescence foci of Cy3 were observed; one of them was localized at high frequency in the cytosol space between the nucleoids, while the other was localized in the cytosol space at a cell pole. These results suggest that replicated DNA molecules of pXX705 were localized randomly in cytosol spaces, but not in nucleoid spaces. Thus, the subcellular distribution of the unstable plasmid pXX705 is markedly different from that of the stable plasmid pXX704 carrying the *sopABC* segment described above.

Discussion

We have succeeded in observing plasmid DNA molecules by the modified FISH method in this study and found that stable mini-F-plasmid molecules having the

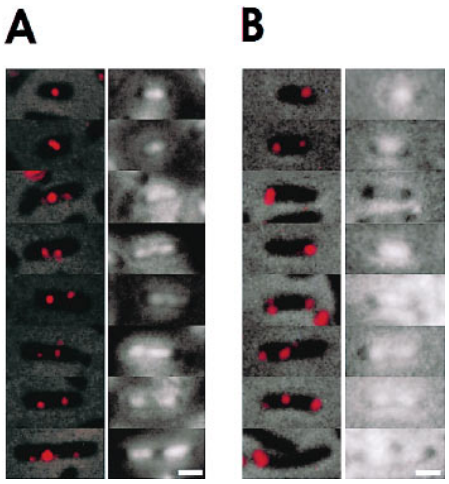

Figure 4. Subcellular Localization of Mini-F-Plasmid DNA Molecules and DAPI-Stained Nucleoids

Combined images of the phase contrast micrograph and fluorescence micrograph. Fluorescence micrographs for Cy3 (left row) and DAPI (right row) in the same field. (A) Cells harboring pXX704. (B) Cells harboring pXX705. Scale bar indicates 1 μm.

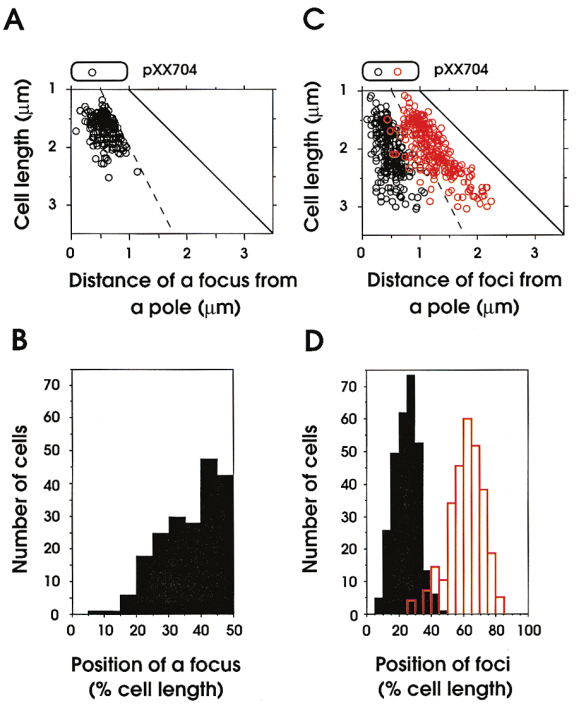

Figure 5. Analysis of Subcellular Distribution of the Stable Plasmid pXX704

Cells harboring pXX704 were fixed and prepared for hybridization with the Cy3-labeled DNA probe.

(A) In cells with one Cy3 fluorescence focus, the distance between the center of the focus and the nearest pole was plotted versus cell length. The broken line indicates midcell and the solid line indicates the position of a pole (n = 200).

(B) Histogram of position of the focus in cells with a single focus. Position of the focus represented a percentage of the cell length (n = 200).

(C) In cells with two Cy3 fluorescence foci, the position of the focus located nearest a pole is plotted as a black circle, and the other focus is plotted as a red circle versus cell length. The broken line indicates midcell and the solid line indicates the position of a pole (n = 280).

(D) Histogram of positions of Cy3 fluorescence foci in cells with two foci. Positions of the foci are represented as black and red bars (n = 280).

sopABC segment are localized at specific cellular sites, the 1/4 and 3/4 positions. In contrast, unstable mini-F-plasmid molecules lacking the *sopABC* segment were randomly localized in cytosol spaces, but not in nucleoid spaces (Figure 7).

As shown in Figure 5C, two plasmid molecules were localized at the 1/4 and 3/4 positions even in some small cells, similar to newborn cells in length. This suggests that when a plasmid molecule is replicated in an early stage of the cell cycle, the resulting molecules migrate at the 1/4 and 3/4 positions without coupling with cell elongation. Furthermore, the frequency of cells with two closely localized plasmid molecules was very small, suggesting that plasmid molecules are positioned at 1/4 and 3/4 positions for a short time after replication. Replicated molecules of the stable mini F plasmid migrate to the 1/4 and 3/4 positions and are tethered there until completion of cell division by a mechanism involving the functions of SopA, SopB, and the *cis*-acting *sopC* site. Therefore, both newborn cells are able to receive at least one plasmid molecule with high fidelity. The present results are inconsistent with models that plasmid molecules are localized at the plane of cell division through septation.

The mini F plasmids replicate once randomly through all stages of the cell cycle (Helmstetter et al., 1997). Therefore, replicated plasmid molecules should move to the cell quarter sites independent of cell elongation. The apparent rapid movement independent of cell elongation is inconsistent with one concept that the migration of replicated molecules to cell quarter sites is coupled with cell elongation (Jacob et al., 1963; Austin and Abeles, 1983). As shown in Figure 5A, there were some long cells having only one focus; perhaps the plasmid did not replicate yet. This is consistent with the plasmid replication through all stages of the cell cycle (Helmstetter et al., 1997). The rapid movement independent of cell elongation probably occurs after replication. Findings of the ATPase activity of SopA homologs (Davis et al., 1992; Watanabe et al., 1992) suggest an energetic movement of plasmids F and P1, as proposed first in the E. coli chromosome (Hiraga et al., 1991).

The tethering of the plasmid to the 1/4 and 3/4 positions probably causes an association with the cell envelope itself or a peripheral structure via the partitioning complex, or partisome (Ogura and Hiraga, 1983b), which involves the plasmid-coded partition proteins and the *sopC* site. Because the 1/4 and 3/4 positions are the future cell division site in the next generation, we presume the possibility that there are specific subcellular structures at the attached sites of the plasmids. The structure may be related to periseptal annuli (de Boer et al., 1990).

In a *minCDE* null mutant producing minicells, P1 and F plasmids having their own partition systems were hardly partitioned to minicells, whereas plasmids lacking the

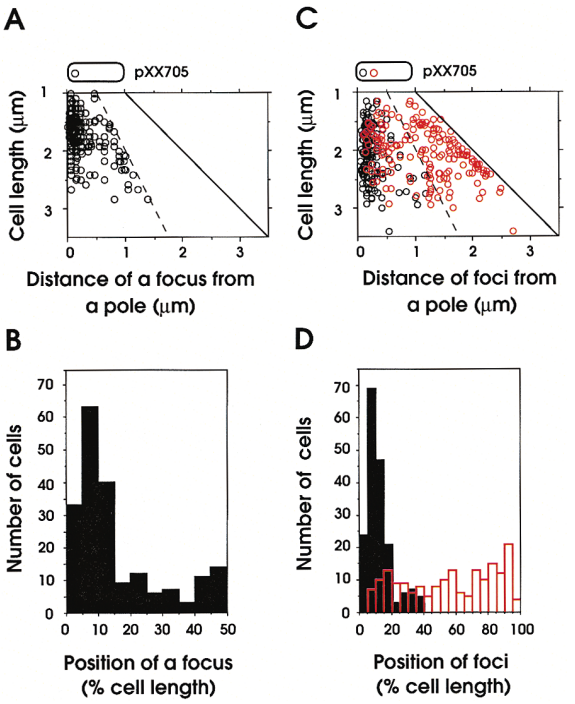

Figure 6. Analysis of Subcellular Distribution of the Unstable Plasmid pXX705

(A) In cells with one Cy3 fluorescence focus, the distance between the center of the focus and the nearest pole is plotted versus cell length. The broken line indicates midcell and the solid line indicates cell length (n = 198).

(B) Histogram of position of the focus in cells with one focus. Position of the focus represented a percentage of the cell length (n = 198).

(C) In cells with two Cy3 fluorescence foci, the position of the focus located nearest a pole is plotted as a black circle, and the other is plotted as a red circle versus cell length (n = 184).

(D) Histogram of positions of Cy3 fluorescence foci in cells with two foci. Positions of foci are represented as black and red bars (n = 184).

partition system were readily partitioned to minicells (Hogan et al., 1982; Eliasson et al., 1992). These results are perfectly consistent with our present observations. Because the minicells are produced near cell poles in the *minCDE* null mutant (de Boer et al., 1989), plasmid molecules lacking the partition system and localized in cytosol spaces of cell poles would be partitioned to minicells. In contrast, plasmid molecules having the partition system are localized at cell quarter positions and therefore cannot be partitioned into minicells.

Homologs of SopA and SopB are involved in the chromosome partitioning in B. subtilis and C. crescentus (Lin et al., 1997; Mohl and Gober, 1997). However, homologs of the *sopA* and *sopB* genes have not been found in the complete genomes of E. coli and H. influenzae. On the other hand, E. coli has the *mukE*, *mukF*, and *mukB* genes, which are essential for chromosome partitioning (Niki et al., 1991; Yamanaka et al., 1996). Homologs of these genes have also been found in H. influenzae, but not yet in other bacteria, including B. subtilis and C. crescentus. *sopA* and *sopB* homologs may not be necessary for chromosome partitioning in E. coli and H. influenzae, because of the presence of *mukF*, *mukE*, and *mukB*.

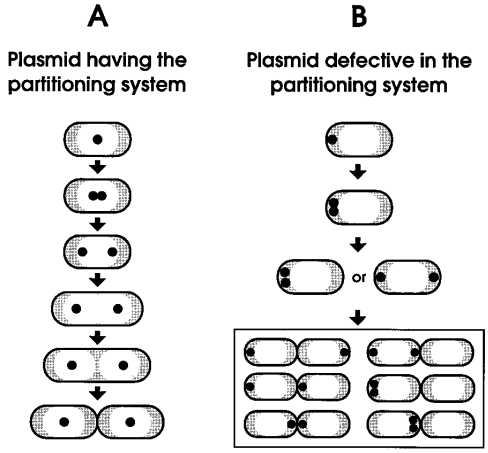

Figure 7. Schema of Subcellular Localization of Plasmids during Cell Division Cycle

(A) DNA molecules of a plasmid having the partitioning system are positioned at the 1/4 and 3/4 positions after replication. The plasmid molecules are tethered in these positions at least until completion of cell division. Septum forms at midcell. Therefore, resulting newborn cells have a plasmid in midcell.

(B) On the other hand, DNA molecules of a plasmid lacking the partitioning system are randomly localized in cytosol spaces, but not in nucleoid spaces. Closed circles indicate plasmid molecules. Open and shaded regions indicate nucleoid and cytosol spaces, respectively.

In the case of the bacterial chromosome, a specific DNA region(s) to which chromosomal partitioning proteins bind presumably exists in the chromosome and plays an important role like a eukaryotic centromere for positioning of the replicated daughter chromosome at the 1/4 and 3/4 positions. It is yet unknown how plasmid molecules or chromosomes move respectively in opposite directions and are positioned at the 1/4 and 3/4 cellular positions and how the driving force is generated. Bacterial cells presumably have a primitive mitotic apparatus involving motor proteins and putative cytoskeleton-like filaments (Hiraga et al., 1991; Hiraga, 1992). It is not yet clear whether the SopA protein, belonging to a Mg^{2+}-dependent ATPase family, acts as a force generating protein in plasmid partitioning.

Experimental Procedures

Bacteria, Plasmids, and Culture Conditions

An E. coli K-12 derivative, CSH50 [*ara* (D*lac-pro*) *strA thi*] (Miller, 1972), was used as host strain for plasmids. The stable mini F plasmid pXX704 was a derivative of pXX326 (Niki et al., 1988), and the isogenic unstable mini F plasmid pXX705 was a derivative of pXX327 (Ogura and Hiraga, 1983a) in which the PvuI–SalI DNA segment containing *sopA*, *sopB*, and *sopC* was deleted. The precise construction of plasmid pXX704 was described previously (Niki et al., 1988). The EcoRI–BamHI segment containing the *lacI* gene derived from pXX703 was ligated with pXX327 (Niki et al., 1988) digested with EcoRI and BamHI, yielding pXX705. Both plasmids pXX704 and pXX705 lack the *ccdA* and *ccdB* genes, responsible for postsegregational killing of plasmid-free segregants (Jaffé et al., 1985); plasmid-free segregants can therefore grow in nonselective medium without ampicillin. The cells were cultivated at 37°C in M9 medium (Miller, 1972) supplemented with glucose (0.5%), proline (50 mg/ml), and thiamine (2 mg/ml) for FISH. Under growing conditions, the doubling time of CSH50 cells was 55 min. Ampicillin (20 mg/ml) was

added to the medium if necessary. To test plasmid stability, plasmid-harboring cells were grown in L medium (1% Bactotryptone, 0.5% yeast extract, 0.5% NaCl [pH 7.4]) at 37°C, and cells maintaining the plasmid were tested as described previously (Niki et al., 1988).

Probes for In Situ Hybridization

pXX704 DNA was used as a probe to observe the subcellular localization of plasmid DNA molecules. Plasmid DNA was purified by the alkaline method and digested with BamHI. After electrophoresis of the digested DNA, the linear plasmid DNA fragment (13.5 kb) was recovered from a low melting point agarose gel. Cy3-dCTP (Amersham) and fluorescein-11-dUTP (Amersham) were used as labeling substrates and incorporated into the template pXX704 DNA fragment using the random-primed DNA labeling kit (Boehringer). After removing nonincorporated substrates by ethanol precipitation, the labeled probe DNA was resolved in hybridization buffer (50% formamide, 2 × SSC, 100 mg/ml salmon testes DNA, 70 mg/ml chromosomal DNA from strain CSH50). The hybridization mixture was sonicated for 15 sec and heated at 75°C for 10 min before hybridization.

Fluorescence In Situ Hybridization

To fix cells, an equal volume of fixation solution (methanol:acetic acid [3:1]) was added directly to a bacterial culture growing exponentially in M9 medium without ampicillin at 37°C. After 5 min at room temperature, the cells were collected by centrifugation and resuspended in 1 ml of fixation solution. The fixed cells in the solution can be kept at 4°C for a few months. On a poly-L-lysine–coated glass slide, 10 μl of the fixed cell suspension was spread and dried at room temperature. To denature DNA of the cells, the sample slide was incubated in denaturing solution (70% formamide, 2 × SSC) at 75°C for 2 min and transferred to prechilled (−35°C) 70% ethanol and kept for 5 min. The slide was transferred into a series of ethanol baths (90% and then 100%, for 5 min each) and dried. The slide was covered with freshly prepared lysozyme solution (25 mM Tris–HCl, 10 mM EDTA, 50 mM glucose, 2 mg/ml lysozyme [pH 8.0]) and kept for 10 min at room temperature. The slide was washed in PBS (80 mM Na_2HPO_4, 20 mM NaH_2PO_4, 100 mM NaCl [pH 7.5]) for 5 min and transferred into a series of ethanol baths (70% and then 100%, for 5 min each) and dried. The slide was incubated overnight at 42°C with hybridization buffer containing the denatured probe labeled with Cy3-dCTP described above. After hybridization, the slide was washed in wash buffer (50% formamide, 2 × SSC) at 37°C for 15 min. The slide was washed with a series of SSC solutions (2×, 1×, 4×, and then 2×, for 5 min each) at room temperature. The slide was washed in PBS containing 10 mM EDTA for 5 min and finally in distilled water for 5 min. The slide was dried and mounting medium (90% glycerol, 1 mg/ml p-phenylenediamine dihydrochloride, 0.15 mg/ml DAPI) was applied.

Microscopy and Image Analysis

All images were recorded with a cooled CCD camera, C5810-01 (Hamamatsu, Japan), using a phase contrast and fluorescence microscopy system (Nikon). The images were directly transferred to a Power Macintosh and processed using Adobe Photoshop 4.0-J software. The image was printed on a Pictrography 300 (Fuji).

Acknowledgments

Correspondence regarding this paper should be addressed to H. N. (e-mail: niki@gpo.kumamoto-u.ac.jp). We thank Drs. R. D'Ari, T. Ogura, and K. Nordström for critically reading the manuscript and comments, and Dr. H. Sasaki for technical advice. This work was supported by a Grant-in-Aid for Scientific Research on Priority Areas, a grant for International Scientific Research Programs for Joint Research from the Ministry of Education, Science, Sports and Culture of Japan, and a grant from the Human Frontier Science Program (contact no. RG-386/95M).

Received June 12, 1997; revised July 11, 1997.

References

Austin, S., and Abeles, A. (1983). Partition of unit-copy miniplasmids to daughter cells. II. The partition region of miniplasmid P1 encodes an essential protein and a centromere-like site at which it acts. J. Mol. Biol. *169*, 373–387.

Austin, S., and Nordström, K. (1990). Partition-mediated incompatibility of bacterial plasmids. Cell *60*, 351–354.

Davis, M.A., and Austin, S.J. (1988). Recognition of the P1 plasmid centromere analog involves binding of the ParB protein and is modified by a specific host factor. EMBO J. *7*, 1881–1888.

Davis, M.A., Martin, K.A., and Austin, S.J. (1992). Biochemical activities of the ParA partition protein of the P1 plasmid. Mol. Microbiol. *6*, 1141–1147.

Davis, M.A., Radnedge, L., Martin, K.A., Hayes, F., Youngren, B., and Austin, S.J. (1996). The P1 ParA protein and its ATPase activity play a direct role in the segregation of plasmid copies to daughter cells. Mol. Microbiol. *21*, 1029–1036.

de Boer, P.A.J., Cook, W.R., and Rothfield, L.I. (1990). Bacterial cell division. Annu. Rev. Genet. *24*, 249–274.

de Boer, P.A.J., Crossley, R.E., and Rothfield, L.I. (1989). A division inhibitor and a topological specificity factor coded for by the minicell locus determine proper placement of the division septum in E. coli. Cell *56*, 641–649.

Eliasson, A., Bernander, R., Dasgupta, S., and Nordström, K. (1992). Direct visualization of plasmid DNA in bacterial cells. Mol. Microbiol. *6*, 165–170.

Ezaki, B., Ogura, T., Niki, H., and Hiraga, S. (1991). Partitioning of a mini-F plasmid into anucleate cells of the *mukB* null mutant. J. Bacteriol. *173*, 6643–6646.

Funnell, B.E. (1988). Mini-P1 plasmid partitioning: excess ParB protein destabilizes plasmids containing the centromere *parS*. J. Bacteriol. *170*, 954–960.

Funnell, B.E., and Gagnier, L. (1995). Partition of P1 plasmids in Escherichia coli *mukB* chromosomal partition mutants. J. Bacteriol. *177*, 2381–2386.

Hanai, R., Liu, R., Benedetti, P., Caron, P.R., Lynch, A.S., and Wang, J.C. (1996). Molecular dissection of a protein SopB essential for Escherichia coli F plasmid partition. J. Biol. Chem. *271*, 17469–17475.

Helmstetter, C.E., Thornton, M., Zhou, P., Bogan, J.A., Leonard, A., and Grimwade, J.E. (1997). Replication and segregation of a miniF plasmid during the division cycle of Escherichia coli. J. Bacteriol. *179*, 1393–1399.

Hiraga, S. (1992). Chromosome and plasmid partition in Escherichia coli. Annu. Rev. Biochem. *61*, 283–306.

Hiraga, S., Niki, H., Imamura, R., Ogura, T., Yamanaka, K., Feng, J., Ezaki, B., and Jaffé, A. (1991). Mutants defective in chromosome partitioning in E. coli. Res. Microbiol. *142*, 189–194.

Hogan, J.E., Kline, B.C., and Levy, S.B. (1982). Regions on the F plasmid which affect plasmid maintenance and the ability to segregate into Escherichia coli minicells. Plasmid *8*, 36–44.

Ireton, K., Gunther, N.W., IV, and Grossman, A.D. (1994). *spo0J* is required for normal chromosome segregation as well as the initiation of sporulation in Bacillus subtilis. J. Bacteriol. *176*, 5320–5329.

Jacob, F., Brenner, S., and Cuzin, F. (1963). On the regulation of DNA replication in bacteria. Cold Spring Harbor Symp. Quant. Biol. *28*, 329–348.

Jaffé, A., Ogura, T., and Hiraga, S. (1985). Effects of the *ccd* function of the F plasmid on bacterial growth. J. Bacteriol. *163*, 841–849.

Kline, B.C. (1985). A review of mini-F plasmid maintenance. Plasmid *14*, 1–16.

Kusukawa, N., Mori, H., Kondo, A., and Hiraga, S. (1987). Partitioning of the F plasmid: overproduction of an essential protein for partition inhibits plasmid maintenance. Mol. Gen. Genet. *208*, 365–372.

Lin, D.C.-H., Levin, P.A., and Grossman, A.D. (1997). Bipolar localization of a chromosome partition protein in Bacillus subtilis. Proc. Natl. Acad. Sci. USA *94*, 4721–4726.

Miller, J.H. (1972). Experiments in Molecular Genetics (Cold Spring Harbor, New York: Cold Spring Harbor Laboratory).

Mohl, D.A., and Gober, J.W. (1997). Cell cycle–dependent polar localization of chromosome partitioning proteins in Caulobacter crescentus. Cell *88*, 675–684.

Mori, H., Kondo, A., Ohshima, A., Ogura, T., and Hiraga, S. (1986). Structure and function of the F plasmid genes essential for partitioning. J. Mol. Biol. *192*, 1–15.

Mori, H., Mori, Y., Ichinose, C., Niki, H., Ogura, T., Kato, A., and Hiraga, S. (1989). Purification and characterization of SopA and SopB proteins essential for F plasmid partitioning. J. Biol. Chem. *264*, 15535–15541.

Motallebi, V.M., Rouch, D.A., and Thomas, C.M. (1990). A family of ATPases involved in active partitioning of diverse bacterial plasmids. Mol. Microbiol. *4*, 1455–1463.

Mysliwiec, T.H., Errington, J., Vaidya, A.B., and Bramucci, M.G. (1991). The Bacillus subtilis *spo0J* gene: evidence for involvement in catabolite repression of sporulation. J. Bacteriol. *173*, 1911–1919.

Niki, H., Ichinose, C., Ogura, T., Mori, H., Morita, M., Hasegawa, M., Kusukawa, N., and Hiraga, S. (1988). Chromosomal genes essential for stable maintenance of the mini-F plasmid in Escherichia coli. J. Bacteriol. *170*, 5272–5278.

Niki, H., Jaffé, A., Imamura, R., Ogura, T., and Hiraga, S. (1991). The new gene *mukB* codes for a 177 kd protein with coiled-coil domains involved in chromosome partitioning of E. coli. EMBO J. *10*, 183–193.

Nordström, K., and Austin, S.J. (1989). Mechanisms that contribute to the stable segregation of plasmids. Annu. Rev. Genet. *23*, 37–69.

Ogasawara, N., and Yoshikawa, H. (1992). Genes and their organization in the replication origin region of the bacterial chromosome. Mol. Microbiol. *6*, 629–634.

Ogura, T., and Hiraga, S. (1983a). Mini-F plasmid genes that couple host cell division to plasmid proliferation. Proc. Natl. Acad. Sci. USA *80*, 4784–4788.

Ogura, T., and Hiraga, S. (1983b). Partition mechanism of F plasmid: two plasmid gene-encoded products and a cis-acting region are involved in partition. Cell *32*, 351–360.

Scott, J.R. (1984). Regulation of plasmid replication. Microbiol. Rev. *48*, 1–23.

Watanabe, E., Inamoto, S., Lee, M.H., Kim, S.U., Ogua, T., Mori, H., Hiraga, S., Yamasaki, M., and Nagai, K. (1989). Purification and characterization of the *sopB* gene product which is responsible for stable maintenance of mini-F plasmid. Mol. Gen. Genet. *218*, 431–436.

Watanabe, E., Wachi, M., Yamasaki, M., and Nagai, K. (1992). ATPase activity of SopA, a protein essential for active partitioning of F plasmid. Mol. Gen. Genet. *234*, 346–352.

Webb, C.D., Teleman, A., Gordon, S., Straight, A., Belmont, A., Lin, D. C.-H., Grossman, A.D., Wright, A., and Losick, R. (1997). Bipolar localization of the replication origin regions of chromosomes in vegetative and sporulating cells of B. subtilis. Cell *88*, 667–674.

Yamanaka, K., Ogura, T., Niki, H., and Hiraga, S. (1996). Identification of two new genes, *mukE* and *mukF*, involved in chromosome partitioning in Escherichia coli. Mol. Gen. Genet. *250*, 241–251.